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Description

The present invention relates to a device and a method for the detection of human chorionic gonadotropin (hCG) in urine for the early pregnancy diagnosis.

Human chorionic gonadotropin (hCG) is a glycoprotein hormone synthetized by the placenta and released in blood and urine soon after the implantation of a fertilized ovum in the chorionic tissue.

Concentration of hCG in urine increases from undetectable levels to 50 International Units/1 2-3 weeks after conception and up to 100.000 IU/l after 10 weeks (Speroff L., Glass R.H., Kase N.G.. In "Clinical Gynecologic Endocrinology and Infertility, 3rd ed., Williams and Wilkins Co., Baltimore, 1983:555). For this reason urinary hCG is generally considered a specific marker of pregnancy.

The diagnosis of pregnancy as early as possibile is particularly important specially when women are exposed, for therapeutic or professional reasons, to chemical or physical agents, or when, for other health safety reason, the pregnancy should be interrupted.

Particular efforts were addressed in the last 20 years to develop simple tests to allow also unskilled people to make their own pregnancy diagnosis at home, thus avoiding psychological and practical problems connected with bringing urine samples to diagnostic laboratories soon after the missed menses period.

Since the risk of making errors by untrained users increases with the number of operations and reagents needed, it is highly desirable to make available pregnancy tests simpler than those so far described. The ideal test should be characterized by an analytical which device is simply dipped into urine, without sampling and pipetting operations or time-fixed incubations, followed by a rapid and inequivocal visual reading of results, preferably in the form of plus/minus signs, including positive and negative controls of reagents, with sufficient sensitivity to detect hCG levels already after one-two days after the missed menses period (50 IU/L). At the present time several types of pregnancy tests have been described and/or marketed but none completely fulfills the need of an ideal home test. Moreover, it should be kept in mind that though the accuracy of the assays described so far is generally claimed to be superior to 99%, when carried out by trained laboratory personell, this could not be true when the assay is performed at home by untrained users. (J.N. Hicks, Iosefsohn M. Clinical Chemistry Vol. 34, 1182 (1988)).

Pregnancy detection methods which are now used, are in large part based on ELISA techniques. This kind of analysis is generally based on a bioselective adsorption on a solid phase coated with anti-hCG antibodies (plastic tubes, balls, rods, membranes etc.) of urine samples previously mixed and incubated with other anti-hCG antibodies labelled with enzyme probes or labels. The immunological reaction is then developed, after appropriate washings, by an enzymatic reaction with chromogen substrates. Although immunoenzymatic tests are generally very sensitive and rapid, they require several operations.

Moreover some of these need particular care, e.g. the washing steps, which can cause "false positives" if not correctly done. Moreover the tendency of the enzyme probe or label to inactivation during storage and analysis may represent another potential source of errors in immunoenzymatic tests.

EP-A-0 243370 discloses immunoenzymatic assays based on particular devices able to help the user: yet some of the above mentioned disadvantages are still present (colors involving error or confusion possibilities in the interpretation of the results, lack of positive and negative reagent controls, impossibility to keep the analysis results for a comparison with the tests carried out subsequently etc.).

Other types of immunoassay have been reported so far having different advantages and disadvantages with respect to ELISA techniques. Great value was shown by assays relying on the use of immunoreactants labelled with directly visible colored cells or particles without extra enzymatic reactions. First to be developed as visually detectable labels were red blood cells used in hCG haemagglutination tests, introduced in the early 60's by Wide and Gemzell (Wide L., Gemzell C.A. Acta Endocrinol. 35, 261 (1960); Wide L. Acta Endocrinol. 70, 1-111, (1962). This test is based on the agglutination of sheep hCG-coated red blood cells caused by immunoreaction with rabbit anti-hCG antiserum. The inhibition of this reaction by the hCG present in the urine sample is indicated by characteristic ring pattern at the bottom of the test tube. Similar methods, based on the use of latex particles are described, for example, in Israel Patent No 50929 and Israel Patent No 47223.

Other direct reading methods so far described rely on the use of antibodies labelled with stained bacteria (EP-A- 0074520), with colloidal metals (Leuvering J.H.W. et Al., J. Immunoassay 1, 77 (1980)) or hydrophobic organic dyes (EP-A No 0032270). Some of these "direct reading" assays are of great value for home pregnancy tests since fulfill the need of maximum semplicity (one step without washings) and good sensitivity, but they still suffer of a number of shortcomings such as the requirement of long fixed time incubations, sampling operations, the need of color estimation or comparison that is often difficult for untrained users (positive and negative results appearing as plus/minus signs are by far more inequivocal and preferable) and do not provide a built-in positive and negative control of reagents in the same assay

device.

Most of these shortcomings have been overcome by the colloidal gold membrane assay (COGMA) described in EP-A-0258963 and EP-A-0254081. This assay combines the rapidity of membrane immunoconcentration ELISA techniques with the simplicity of assays relying on visually detectable probes (no need of washing steps and chromogenic reactions). As described for example in EP-A-0254081, pregnancy tests can be carried out in a few minutes with four operations: 1) the urine sample to be tested is pipetted into a filtration apparatus located above a membrane carrying an anti-hCG antibody, 2) the urine sample is left to absorb through the filter and membrane by means of a physically absorbent material contacted with the lower part of the membrane, 3) the filtration apparatus is removed, 4) a second gold labelled anti-hCG antibody is then pipetted and left to absorb through a membrane. A reddish spot is observed immediately at the center of the membrane when urine contains no more than 5 IU/l of hCG.

EP-A-0258963 describes a pregnancy test completely similar in principle to that described in EP-A 0254081 except for the final results which are obtained in a more inequivocal form of plus/minus signs.

EP-A-0250137 describes a different form of colloidal gold membrane assay for quantitative measurement of various analytes, also appliable for pregnancy detection. The sample and the gold-labelled reagent are mixed and then contacted with one end of a membrane strip carrying the binding reagent as horizontal band and allowing upward flow through a membrane. The height of the visually observable color may be correlated to the presence of the ligand to be determined in the fluid sample. However, though this assay gives quantitative information, it does not seem to provide remarkable advantages for home qualitative hCG tests and more important, the results are not obtained as plus/minus spots which are preferable for unskilled users.

Though the colloidal membrane assays represent a remarkable advantage in home pregnancy test they do not completely fulfill the need of an ideal test which should not involve pipetting, urine sampling and filtering operations.

The use of reagent impregnated test-strips in specific binding assays, has previously been proposed, for example, in EP-A-0225054 (WO-A-8702774), US-A-4 624 929, EP-A-0217403 and EP-A-0291194.

It is an object of the present invention to provide an analytical device and method for a more simpler and reliable pregnancy detection by unskilled users. The method and the device are characterized by the following adavantages over the previously described assays and methods:

a) they do not require filtering operations;
b) they do not require pipetting operation;
c) they do not require time measurements;
d) they do not require exact volume measurements;
e) they do not require washing steps;
f) they require only one simple dipping operations;
g) they are rapid (require no more than 3-5 minutes);
h) they have a high detectability (detection limit, 100 U/l);
i) they do not contain additional liquid reagents, avoiding any possible confusion;
l) they allow long storage of results without bleaching allowing comparisons with tests carried out afterward;
m) they provide a built-in positive and negative control of reagents and assay performance;
n) they allow inequivocal visual reading of results in the form of plus/ minus signs.

No assay method so far described present all of these properties combined in the same analytical system. The above mentioned effects are obtained thanks to the device and the method of the invention, based on the principle of sandwich reactions between hCG an a couple of biospecific hCG binding reagents, one of which is immobilized in a particular part of the reading area of the device and the other labelled with visually detectable compound or particle. The device according to the invention comprises:
a first container having distal and proximals ends, said distal end containing an entrance through which the body fluid is capable of being drawn,
a second container having distal and proximal ends, and said second container being housed within and capable of being removed from said first container,

a) a first area, consisting of a first material contained in said first container within an area defined between the distal end of the first and the distal end of the second container, said first material capable of drawing a body fluid by capillarity and on which a color labelled first protein capable of specifically binding to said analyte is absorbed,

b) a reading area, consisting of a second material contained within the distal end of said second container comprising a reading area in flat contact with said first material and on said reading area is bound

(i) a second protein capable of specifically binding to the analyte
(ii) the analyte and
(iii) an inert protein,

wherein (i), (ii) and (iii) are disposed on said second material so as to form, after reaction with said first color labelled protein, at least one or two figures which can be visually detected when the second container is removed from the first container,

a third material capable of drawing the body fluid by capillarity contained within said second container and contacting said second material, and

a flow indicator means contained within said third material, said means being capable of indicating the passage of a body fluid through said device.

The areas b) and c) together constitute an element which can be called "immunoconcentrator" element while the a) area constitutes a compartment wherein the bioselective colored reagent is present in a preabsorbed state on an appropriate supporting material, from which it is made available by the capillary flow of urine under exam.

According to the present invention the colored reagent comprises an hCG-biospecific binding reagent able to form sandwich complexes with hCG and other hCG-binding reagents immobilized on the reading area, labelled with a visually detectable compound, particle, or cell. Examples of hCG binding reagents which can be used in a labelled forma are monoclonal and polyclonal antibodies or lectins (Concanavalin A, wheat germ lectin, lentil lectin and soy bean lectin) selected for their capability of making sandwich complexes with hCG and other hCG-binding reagents immobilized onto the reading area, e.g. by binding to different epitopes or sites on the hCG molecules. Several methods are known for labelling immunoreactants with visually detectable tags. This type of label may include stained bacteria (e.g. killed stained Staphylococci), colored latex particles, hydrophobic dyes, colloidal metals able to bind proteins when adjusted to the optimal pH and concentration (gold, silver, platinum, copper, and the metal compounds sodium hydroxide, silver iodide, silver bromide, copper hydroxide, aluminium hydroxyde, chromium hydroxide, vanadium oxide, arsenic iodide, manganese hydroxyde and the like). Methods for coupling colored particles to proteins are already known and well described in the above cited references.

Preferred colored tag for immunoreactants in the present invention are colored particles with a diameter size lower then 200 nm.

The preferred bioselective colored reagents are monoclonal or polyclonal anti-hCG antibodies labelled with colloidal gold, recently commercially available. The size of the colloidal metal particle is preferably from 3 to 100 nm.

Said colored bioselective reagent is adsorbed on a supporting material which has capillary properties and is able to assure the eluition of the colored bioselective reagent thanks to the action of the capillary forces at the urine passage. A cellulose or cellulose powder absorbing filter is useful for this purpose.

The reading area consists of a membrane or filter able to bind proteins, such as nitrocellulose, nylon, immunodyne, biodyne, cyanogen bromide, activated paper with pore size ranging from 0.45 to 12 μm, preferably from 0.45 to 1.2 μm.

Examples of hydrophylic capillary materials are paper, cellulose powder cotton or other cellulose derivatives, hydrophylic polymers, polysaccharides or polyols, kaolin, titanium dioxide, barium sulfate, diatomaceous earth.

The flow indicator which is present on the upper part of said material is, for instance, a pH indicator compound able to change color when wetted by urine, for instance bromophenol blue. The device of the invention can be shaped in several forms suited for the intended use, for instance as a stick, small tube, strip-supported on plastic material, paper or the like.

In the preferred embodiments of the invention the "reading area" consists of a piece of 0.5-2 cm^2 of commercially available filter paper containing nitrocellulose or other mechanically strong protein binding membrane, contacted with the lower part of a cellulose absorbent stick, containing in the upper part a pH-indicator compound. The nitrocellulose filter and the absorbent stick are tightly sealed with a plastic material leaving a part of the nitrocellulose filter free to contact the area a). Also the upper part of the immunoconcentrator stick is left free.

hCG, an anti-hCG mono or polyclonal antibody and an inert protein, as described herinafter, are bound on the nitrocellulose filter. The stick is then introduced in an appropriate container, in which the biospecific colored reagent absorbed at a dry state on a cellulose material, is contained, so that the visually detectable nitrocellulose membrane contacts it.

This nitrocellulose membrane is structurally divided in three areas: the first area (herein after referred as I-SA) carries immobilized hCG; the second area (herein after referred as II-SA) carries an anti-hCG monoclonal or polyclonal antibody; and the third area (herein after referred as III-SA) carries an inert protein,

that is bovine serum albumine.

Alternatively other proteins can be used in the three areas: for example hCG-binding lectins can be used instead of monoclonal or polyclonal antibodies in II-SA; casein, fatty acid free milk, ovalbumin, gelatin, non immune sera or any other inert protein can be immobilized instead of bovine serum albumine in III-SA. The proteins can be immobilized according to methods well known to the man skilled in the art or by following the membrane manufacturer's instructions. Protein solutions can be simply dropped onto the relevant area or sprayed in narrow bands using a TLC quantitative sample applicator.

The invention is now described with reference to the enclosed drawings, wherein:

Figure 1a shows an embodiment of the device of the invention as seen in a longitudinal section.

Figure 1b is an exploded view of the device of Figure 1a.

Figure 1c is a view from the top of the nitrocellulose filter which constitutes the reading area of the device.

In the above mentioned Figures, the reference number 1 shows the nitrocellulose filter, while numbers 1A, 1B and 1C show the areas I-SA, II-SA and III-SA respectively; the reference number 2 indicates the hydrophilic material which assures the capillary flow of urine, while the reference 3 shows the area of the same material 2 on which a pH indicator was left to adsorb. Number 4 indicates the plastic envelope constituting the stick. Number 5 shows an opened, inert element which closes the upper part of the device.

Finally, number 6 shows the container where the colored biospecific reagent, adsorbed on cellulosic material 7, is present. The container number 6 presents an hydrophilic pore sectum, which closes a hole of container 6 to be dipped in the urine sample when the analysis is carried out.

Therefore the only requested operations are the immersion of the device in urine until area 3 changes color, showing that the device was filled with urine, and the following removal of the stick with a visual inspection of the nitrocellulose membrane.

Results are read as follows:

- staining of II-SA is indicative of pregnancy; unstaining is indicative of non-pregnancy
- I-SA and II-SA are control areas for reagents and assay performance. In particular I-SA must always stain (positive control) while III-SA must never stain (negative control). Preferred forms of the final colored signals are a plus sign (+) for pregnancy and a minus sign (-) for non pregnancy. This can be obtained for example by dividing the reading areas in I-SA, II-SA, and III-SA as depicted in fig. 1. I-SA (positive control, horizontal tract) always stains independently on the presence of hCG in urine, II-SA (test area, vertical tract) stains only when hCG is present in urine; III-SA (negative control, sorrounding area) never stains independently of the presence of hCG in urine.

The invention also refers to a method for the determination of hCG in urine, in which urine is contacted with:

a) an hCG bioselective colored reagent adsorbed at dry state on a material which allows the capillary flow of urine;

b) a membrane of nitrocellulose or other material able to strongly bind proteins on which a protein able to selectively bind hCG and possibly hCG and a different protein are irreversibly bound;

c) an hydrophilic material on which a pH indicator is preferably adsorbed.

The method which is object of the present invention is based, in detail, on the use of the device described hereinbefore, which allows the pregnancy analysis with just one dipping operation and a subsequent direct and clear reading of the results. The method and the device of the invention allow the pregnancy analysis in few minutes and with a sensitivity equal to that of the best systems available up to now. The device of the invention can be part of a kit which, for instance, consists in a urine container endowed with a support for the analytical device. Moreover, this kit has instructions to carry out pregnancy analysis in accordance with the invention.

Though the device and method of the present invention are preferably used in the determination of hCG in urine, they can easily be adapted to the use in the determination of other analytes, especially other immunologically reactive analytes, and in other body fluids, like blood, serum etc. The reagents necessary for the adaptation are known in the state of the art.

The following Example illustrates the present invention in a non-limitative way.

EXAMPLE 1

Materials.

The following materials were used: colloidal gold particles, 15 nm, (Janssen Life Sciences Products, Olen, Belgium), nitrocellulose, 0.45 µm, supported on filter paper (1 mm thick) (Chemetron Laboratories,

Milan, Italy); human chorionic gonadotropine, 5000 IU/mg, (Prodas s.r.l., Milan, Italy); bovine serum albumine (Sigma Chemical Co., St Louis, MO 63178); cellulose powder; anti-hCG (β-subunit) monoclonal antibodies Mab B2, Mab A1 and Mab C3 were obtained by Boehringer Mannheim Milano, Italy.

All the other reagents were analytical grade products by Merck (Darmstad, FRG) or Carlo Erba (Milano, Italy).

Preparation of the analytical device.

A) Preparation of immunoconcentrator deep-stick.

Round pieces of nitrocellulose filters (0.9 cm, diameter) were washed for 10 minutes with distilled water and air dried for 15 minutes. Then 3 µl aliquotes of a 0.1 mg/ml hCG solution (5000 IU/mg) in 3 mM potassium chloride, 136 mM sodium chloride, 10 mM sodium phosphate buffer (PBS), pH 7.4 were applied onto the filter surface by using particular plastic applicators prepared as follows: polystirene plastic sticks (80 mm x 1 mm x 7 mm) were carved longitudinally on the base (0.3 mm deep) and slightly dipped into the hCG solution. About 3 µL of hCG were withdrawn by capillary forces in the carved surface of each plastic stick. Then the withdrawn solutions were dot blotted onto the nitrocellulose filters simply by touching the filters with the plastic sticks. Since the binding is rapid, the hCG spots on each filter resulted as rectangles of about 7 mm x 1 mm. The filters were washed twice with PBS and air dried. Similarly 2 µl aliquotes of a 1 mg/ml Mab B2 solution in PBS were applied to each piece of nitrocellulose filter forming a cross with the "hCG area", as described in fig. 1. The filters were left to dry for 15 min and blocked by incubation for 30 min with a 10% BSA solution in PBS at room temperature. Then each filter was washed twice with PBS, left to dry for at least 15 min. In parallel, the top of cellulose cilindric absorbent filters (diameter, 0.9 cm; height, 10 cm) was dipped into bromophenol blue solutions in 0.01 M HCl and left to absorb and dry at room temperature. This compound, yellowish under these conditions, turns blue when the filter is wetted by urine during the assay.

After drying, both nitrocellulose membranes and absorbent filter were assembled in a cilindric transparent polyethylene plastic container (internal diameter 0.9 cm, height 10 cm) as depicted in fig. 1. The filters were pressed in order to ensure complete and uniform contact between nitrocellulose filter and absorbent cellulose filter and fixed on the top with a plugs having a hole (1 mm, diameter) in the center, to ensure air outlet and the capillary upflow of urine during the pregnancy test.

B) Preparation of gold-labelled anti-hCG antibodies

Compartment.

The anti-hCG Mab C3, Mab B2, Mab A1 were labelled with colloidal gold particles of 15 nm, at pH 6, according to the manufacturer's instructions (Janssen Life Products, Beerse, Belgium). The final products were centrifuged for 30 min at 12,000 g and resuspended in 0.15 M sodium chloride, 0.02 M TRIS-HCl buffer, pH 8.2, containing 200 g/L glycerol, 10 g/L BsAand 20 mM sodium azide, diluted with the same buffer at 4.0 Optical Density units (520 nm), and kept a 4 °C until use.

The various gold-labelled monoclonal antibodies were tested for: a) their capability to form visually detectable sandwich complexes with hCG (0.5 U) and Mab B2 antibody immobilized on the nitrocellulose filters as described above; b) binding to hCG directely immobilized on the nitrocellulose filter; c) binding to nitrocellulose blocked with BSA. The results showed that sandwich complexes with hCG and Mab B2 were obtained with all gold labelled antibodies. However Mab AC-Gold was unable to bind hCG directely immobilized on nitrocellulose and because the most visually detectable signal was obtained with Mab C3-Gold conjugate. Thus this gold labelled antibody was selected for the preparation of the "gold labelled antibody compartment" of the device.

0.5 g of cellulose powder portions were presaturated for 2 h at 37 °C with 1% BSA and washed twice with TBS by repeated centrifugation and resuspension. Then the powder was dried at 37 °C, mixed with 0.5 ml aliquotes of Mab C3 gold conjugate diluted 1:10 with TBS, and dried again at 37 °C. The powder obtained was used to fill a polyethylene plastic tube having a filter on the bottom.

The immunoconcentrator deep-stick and the gold labelled antibody compartment were assembled as depicted in Fig. 1.

C) Detection of hCG in urine

The analytical devices prepared as described above, were dipped in urine or standard samples until the indicator area changed color (about five minutes). Then the immunoconcentrator sticks were removed from the device for inspection of the "reading area". Results, appearing as red plus/minus signs, were visually evaluated.

D) Detectability and accuracy

In order to evaluate the detectability and the accuracy of this assay we have added increasing amounts of hCG to urine samples of non-pregnant women and further analyzed. Urine samples containing more than 100 U/l produced a "plus" sign clearly distinct from the "minus" sign obtained in the absence of hCG. Moreover, when we repeated 10 times the assay of the urine sample containing 100 UI, we obtained in all cases positive results indicating that 100 U/L can be assumed as "cut-off" limit for positive and negative results. In order to further evaluate the accuracy of pregnancy detection, 49 samples of urine from pregnant women and 52 samples from non-pregnant women (total: 101 samples of urine) were tested according to the method of the present invention. As shown in Tab. 1, the results observed were identical to those expected indicating that the assay of the invention is satisfactorily accurate.

Table 1

Detection of hCG in urines from pregnant and non-pregnant women			
Pregnant a) (Positive, N.o)		Non-pregnant a) (Negative, N.o)	
Expected	Observed	Expected	Observed
49	49	52	52

a) Pregnancy was detected in parallel by using the Event Test Color Kit (Boehringer Biochemia Robin, Italy).

Claims

1. A device to detect an analyte in a body fluid, including
a first container (6) having distal and proximal ends, said distal end containing an entrance through which the body fluid is capable of being drawn,
a second container (4) having distal and proximal ends, and said second container (4) being housed within and capable of being removed from said first container(6),
a first material (7) contained in said first container (6) within an area defined between the distal end of the first (6) and the distal end of the second container (4), said first material (7) capable of drawing a body fluid by capillarity and on which a color labelled first protein capable of specifically binding to said analyte is absorbed,
a second material (1) contained within the distal end of said second container comprising a reading area in flat contract with said first material and on said reading area is bound
(i) a second protein capable of specifically binding to the analyte,
(ii) the analyte and
(iii) an inert protein
wherein (i), (ii) and (iii) are disposed on said second material so as to form, after reaction with said first color labelled protein, at least one or two figures which can be visually detected when the second container is removed from the first container,
a third material (2) capable of drawing the body fluid by capillarity contained within said second container and contacting said second material, and
a flow indicator means (3) contained within said third material, said means being capable of indicating the passage of a body through said device.

2. A device according to claim 1, characterized in that the body fluid is urine.

3. A device according to any of claims 1 and 2, characterized in that the analyte is human chorionic gonadotropin (hCG).

4. A device according to claim 3, characterized in that said color labelled first protein and / or said second protein is a monoclonal antibody.

5. A device according to claim 1, characterized in that said first and said third material are cellulosic.

6. A device according to claim 1, characterized in that said second material is a cellulose nitrate filter.

7. A device according to claim 1, characterized in that said at least one or two figures which can be visually detected according to the positivity of the reaction is a plus/minus sign.

8. The method of detecting an analyte in a body fluid, comprising the steps of:
(a) providing a device according to any of claims 1 - 7
(b) contacting the distal end of said device with a fluid sample and allowing said sample to migrate into said device by capillary action thereby subjecting the analyte present in said sample to immunoreaction with said color labelled first protein capable of specifically binding to said analyte to form a labelled immunocomplex, followed by further migration of the immunocomplex onto said second material, wherein said immunocomplex is bound to said second protein capable of specifically binding to the analyte,
(c) removing said second container from said first container, and
(d) visually detecting said at least one or two figures to determine if the analyte is present in said sample.

Patentansprüche

1. Vorrichtung zum Nachweis eines Analyten in einer Körperflüssigkeit, umfassend
einen ersten Behälter (6) mit einem nahen und einem fernen Ende, wobei das ferne Ende einen Einlaß aufweist, durch den die Körperflüssigkeit angesaugt werden kann,
einen zweiten Behälter (4) mit einem nahen und einem fernen Ende, wobei der zweite Behälter (4) innerhalb des ersten Behälters (6) untergebracht ist und aus diesem ersten Behälter (6) herausnehmbar ist,
ein sich innerhalb des ersten Behälters (6), und zwar in einem zwischen dem fernen Ende des ersten Behälters (6) und dem fernen Ende des zweiten Behälters (4) definierten Bereich, befindliches erstes Material (7), das aufgrund von Kapillarkräften in der Lage ist, Körperflüssigkeit anzusaugen und auf dem ein mit einer Farbmarkierung versehenes, den Analyten spezifisch bindendes ersten Protein absorbiert ist,
ein sich innerhalb des fernen Endes des zweiten Behälters befindliches zweites Material (1), das einen mit dem ersten Material in flacher Verbindung stehenden Lesebereich umfaßt, auf dem
(i) ein zweites, den Analyten spezifisch bindendes Protein,
(ii) der Analyt und
(iii) ein inertes Protein
gebunden sind, wobei (i), (ii) und (iii) auf dem zweiten Material so vorgesehen sind, daß nach Reaktion mit dem ersten, farbmarkierten Protein mindestens ein oder zwei Zeichen gebildet werden, die nach Herausnahme des zweiten Behälters aus dem ersten Behälter visuell nachweisbar sind,
ein drittes Material (2), das aufgrund von Kapillarkräften in der Lage ist, das in dem zweiten Behälter befindliche Material anzuziehen und mit dem zweiten Material in Verbindung zu bringen, und
ein sich innerhalb des dritten Materials befindliches Mittel (3) zum Anzeigen eines Fließvorganges, wobei dieses Mittel in der Lage ist anzuzeigen, ob eine Substanz die Vorrichtung durchquert.

2. Vorrichtung nach Anspruch 1, dadurch gekennzeichnet, daß es sich bei der Körperflüssigkeit um Urin handelt.

3. Vorrichtung nach einem der Ansprüche 1 und 2, dadurch gekennzeichnet, daß es sich bei dem Analyten um humanes gonadotropes Chorionhormon (hCG) handelt.

4. Vorrichtung nach Anspruch 3, dadurch gekennzeichnet, daß es sich bei dem ersten farbmarkierten Protein und/oder dem zweiten Protein um einen monoklonalen Antikörper handelt.

5. Vorrichtung nach Anspruch 1, dadurch gekennzeichnet, daß es sich bei dem ersten und dem zweiten Material um ein zelluloseartiges Material handelt.

6. Vorrichtung nach Anspruch 1, dadurch gekennzeichnet, daß es sich bei dem zweiten Material um einen Zellulosenitratfilter handelt.

7. Vorrichtung nach Anspruch 1, dadurch gekennzeichnet, daß mindestens eines der beiden visuell nachweisbaren Zeichen je nach Positivität der Reaktion ein Plus/Minuszeichen ist.

8. Verfahren zum Nachweis eines Analyten in einer Körperflüssigkeit, welches folgende Schritte umfaßt:
   a. das Vorsehen einer Vorrichtung nach einem der Ansprüche 1 - 7,
   b. das Inkontaktbringen des fernen Endes der Vorrichtung mit einer flüssigen Probe, wobei die Probe aufgrund von Kapillarkräften in die Vorrichtung wandern kann und der in der Probe enthaltene Analyt mit dem farbmarkierten ersten Protein, das den Analyten spezifisch bindet, in einer Immunreaktion einen markierten Immunkomplex bildet, und der Immunkomplex danach weiter auf das zweite Material wandert, um dort an das zweite Protein, das den Analyten spezifisch bindet, gebunden zu werden,
   c. das Herausnhemen des zweiten Behälters aus dem ersten Behälter,
   d. der visuelle Nachweis von mindestens einer oder zwei Zeichen, um festzustellen, ob der Analyt in der Probe vorliegt.

Revendications

1. Dispositif pour détecter un analyte dans un fluide corporel incluant:
   - un premier conteneur (6) ayant des extrémités distale et proximale, ladite extrémité distale contenant une entrée à travers laquelle le fluide corporel est capable d'être attirer,
   - un deuxième conteneur (4) ayant des extrémités distale et proximale, et ledit deuxième conteneur (4) étant logé à l'intérieur et capable d'être retiré dudit premier conteneur (6),
   - une première matière (7) contenue dans ledit premier conteneur (6) à l'intérieur d'une zone définie entre l'extrémité distale du premier conteneur (6) et l'extrémité distale du deuxième conteneur (4), ladite première matière (7) capable d'attirer un fluide corporel par capillarité et sur laquelle une première protéine marquée avec un colorant, capable de se lier spécifiquement audit analyte, est absorbée,
   - une deuxième matière (1) contenue à l'intérieur de l'extrémité distale dudit deuxième conteneur comprenant une zone de lecture en contact plat avec ladite première matière et sur ladite zone de lecture sont liées
     (i) une deuxième protéine capable de se lier spécifiquement à l'analyte,
     (ii) l'analyte et
     (iii) une protéine inerte
     dans laquelle (i), (ii) et (iii) sont disposés sur ladite deuxième matière de façon à former, après réaction avec ladite première protéine marquée avec un colorant, au moins une ou deux figures qui peuvent être visuellement détectées quand le deuxième conteneur est retiré du premier conteneur,
   - une troisième matière (2) capable d'attirer un fluide corporel par capillarité contenu à l'intérieur du deuxième conteneur et d'être en contact avec ladite deuxième matière, et
   - un moyen d'indicateur d'écoulement (3) contenu à l'intérieur de ladite troisième matière, ledit moyen étant capable d'indiquer le passage d'un corps à travers ledit dispositif.

2. Dispositif selon la revendication 1, caractérisé en ce que le fluide corporel est de l'urine.

3. Dispositif selon l'une quelconque des revendications 1 et 2, caractérisé en ce que l'analyte est la gonadotropine chorionique humaine (hCG).

4. Dispositif selon la revendication 3, caractérisé en ce que ladite première protéine marquée avec un colorant et/ou ladite deuxième protéine est un anticorps monoclonal.

5. Dispositif selon la revendication 1, caractérisé en ce que ladite première matière et ladite troisième matière sont cellulosiques.

6. Dispositif selon la revendication 1, caractérisé en ce que ladite deuxième matière est un filtre de nitrate de cellulose.

7. Dispositif selon la revendication 1, caractérisé en ce qu'au moins une ou deux figures, qui peuvent être visuellement détectées selon la positivité de la réaction, est un signe plus/moins.

8. Procédé de détection d'un analyte dans un fluide corporel, comprenant les étapes consistant:
(a) à fournir un dispositif selon l'une quelconque des revendications 1 à 7;
(b) à mettre en contact l'extrémité distale dudit dispositif avec un échantillon de fluide et à laisser ledit échantillon migrer dans ledit dispositif par action capillaire, soumettant ainsi l'analyte présent dans ledit échantillon à une réaction immunologique avec ladite première protéine marquée avec un colorant capable de se lier spécifiquement audit analyte pour former un immunocomplexe marqué, suivi par la migration supplémentaire de l'immunocomplexe sur ladite deuxième matière, dans laquelle ledit immunocomplexe est lié à ladite deuxième protéine capable de se lier spécifiquement à l'analyte,
(c) à enlever ledit deuxième conteneur dudit premier conteneur, et
(d) à détecter visuellement lesdites au moins une ou deux figures pour déterminer si l'analyte est présent dans ledit échantillon.

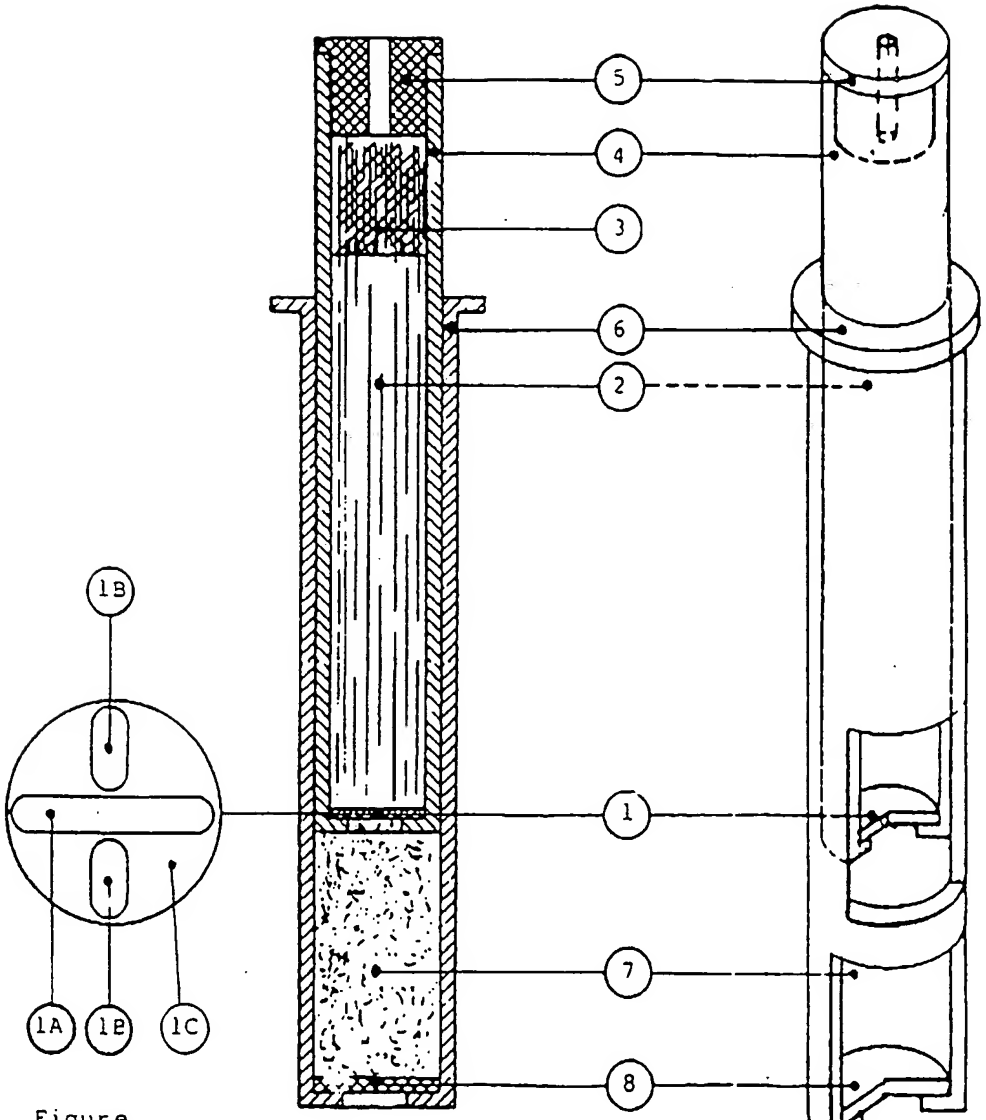

Figure 1c

Figure 1a

Figure 1b